

Advances in Biomagnetic Interfacing Concepts Derived from Polymer-Magnetic Particle Complexes

6/1/03-5/31/04 with a no-cost extension to 12/31/04

Final Report: June, 2005

J.S. Riffle

This final report includes (1) results reported in the interim report submitted in September, 2004, and (2) results from the final three months of research conducted from October through December, 2004. All of the research reported for the October-December, 2004 period relates to the formation of magnetite-poly(lactide) complexes and their assembly into magnetic microspheres.

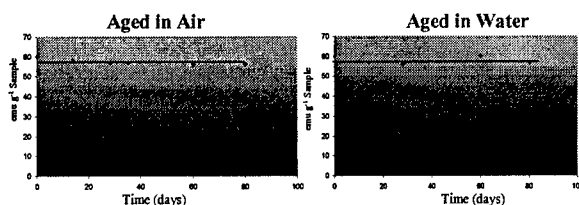
Summary

Our research on the development and characterization of magnetic nanoparticle-polymer complexes for the project period 6/1/03-12/31/04 has yielded approximately 10-nm diameter cobalt particles coated with graphitic or silica protective shells to prevent oxidation of the particle surfaces. The specific saturation magnetizations of the protected particles range from 50-112 emu g⁻¹, which is up to about five times the response of our previous nanoparticle materials. Oxidative durability results demonstrate that both classes of systems yield oxidatively-stable cobalt nanoparticles in air or water. However, the graphitic coatings are significantly more mechanically robust relative to the silica coatings. By contrast, the silica coatings can be readily functionalized with desirable functional groups. Collaborations with Prof. St. Pierre's biophysics group in Australia have been valuable to understand the material and magnetic properties of these new materials.

Accomplishments during this project also include a method for preparing magnetite microspheres with the biodegradable polymer, poly(L-lactide), strongly adsorbed onto the particle surfaces. The magnetite nanoparticles for this research are 4.8 nm in radius (by dynamic light scattering), and are superparamagnetic. Efforts with the magnetite-polymer complexes have been coordinated with Drs. Rosengart (U. Chi.) and Kaminski/Mertz (Argonne), and will continue as we jointly learn how to tailor surfaces to avoid immune response and to complex with biospecific groups. We have focused on binding different amounts of poly(L-lactide) copolymers to the

magnetite nanoparticle surfaces and subsequent characterization of those complexes. We are trying to combine biotin-functional poly(ethylene oxide-*b*-lactide) prepared at Argonne with the magnetite-copolymer complexes prepared at VT to synthesize appropriately sized microspheres for magnetic collection.

Cobalt-silica nanoparticle complexes are oxidatively stable after pyrolysis at 600 °C
(Specific Saturated Magnetization of the Complexes)



16,000 g mol⁻¹ - 3400 g mol⁻¹ PDMS-*b*-[PMVS-*co*-PMTMS]
copolymer/cobalt complexes

Figure 1. Stability in the magnetic properties of cobalt-silica nanoparticle complexes demonstrates the oxidative durability of these materials.

Results of oxidative durability studies on cobalt nanoparticles with protected surfaces.

Cobalt nanoparticles (~10-nm in diameter) have been prepared in our laboratories in several key microphase-separated block copolymer solutions [1-9]. We have discovered two families of block copolymers which can

template cobalt, then can be pyrolyzed at 600-700 °C to form protective ceramic shells around the

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nanoparticles. The ceramic coatings have chemical compositions similar to silica in one case [6, 8], and a graphitic coating in the other [9]. Durability measurements of the specific saturation magnetizations of the cobalt nanoparticles demonstrate that these materials are protected from oxidation by the ceramic shells (figures 1 and 2). The magnetic responses of these materials increase substantially after the heat treatment in terms of the mass specific magnetization of the cobalt, and the Australian collaborators are working to understand this phenomenon. Results show that the crystallinity of the cobalt nanoparticles increases during the heat treatments, and the large increases in magnetization are attributed to that feature. Further tests were conducted to determine whether the mechanical properties of the ceramic shells would be sufficiently robust to withstand intensive milling. It was found that the graphitic coatings withstood such milling processes [9], but the silica coatings were too brittle [8]. Importantly, the saturated responses of these nanoparticles are significantly higher than for currently-available magnetite particles.

“Re-functionalization” of the protected particles to achieve dispersibility in biological media, to provide a means for attaching biospecific groups, and to enable microsphere formation encapsulating therapeutic agents.

After the cobalt particles are formed within the block copolymer templates in solution, these cobalt nanoparticles are heat-treated to produce conformal, protective ceramic shells. A portion of the organic polymer surrounding the particle thermally degrades as the shell forms. Thus, one focus has been to learn how to “re-functionalize” the particle surfaces, so that they can be dispersed in biological fluids, formed into microspheres, and functionalized with biospecific groups. Results demonstrate that the silica-coated cobalt can be re-functionalized with several desired functional groups [8], but we have found it extremely difficult to penetrate the graphitic coatings well enough to functionalize them efficiently. The cobalt nanoparticles with silica-like shells have been re-functionalized with primary amines by coating them with aminopropyltrimethoxysilane and condensing the coupling agent in dry, refluxing toluene. The concentrations of primary amine on the particle surfaces are controllable and agree reasonably well with the charged concentrations (figure

Cobalt nanoparticles encapsulated with pyrolyzed phthalonitrile copolymers are oxidatively stable whereas particles coated with phthalonitrile or cyanate ester networks lack oxidative resistance.

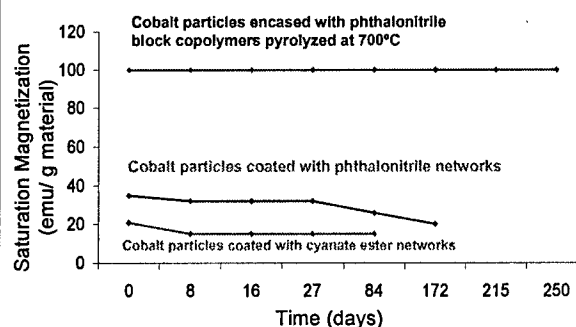


Figure 2. Cobalt nanoparticles encapsulated with pyrolyzed phthalonitrile copolymers are oxidatively stable whereas particles coated with phthalonitrile or cyanate ester networks lack oxidative resistance.

Comparisons of Charged Aminopropylsilane Concentrations with Surface Concentrations Obtained

Co-NH ₂ (mg)	Charged Amine Conc. (mol/g)	Surface Conc. of Amine Obtained (mol/g)
45	0.044	0.036
44	0.044	0.045
48	0.044	0.035
		Ave. 0.038
47	0.024	0.029
45	0.024	0.014
46	0.024	0.022
		Ave. 0.022

Surface amine groups can be quantified by derivatization with trifluoroacetic anhydride, hydrolysis of excess anhydride, then titration of the trifluoroacetic acid with base.

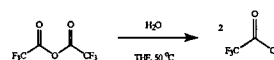
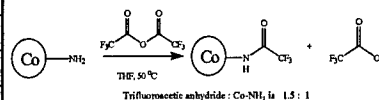
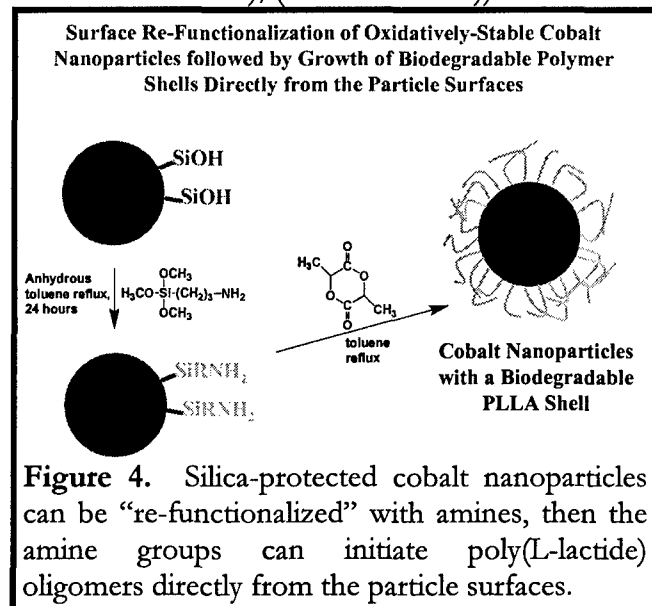


Figure 3. Results demonstrate that the concentration of amines on silica-protected cobalt nanoparticles can be controlled according to charged concentrations of coupling reagents.

3). We have established a method for quantifying the amine groups by derivatizing them with trifluoroacetic anhydride, hydrolyzing excess anhydride, then back-titrating the resultant acids. The surface-tethered amines can initiate L-lactide and the biodegradable polymer, poly(L-lactide), can be polymerized directly from the protected cobalt surfaces (figure 4). It is important that the concentration of surface amines be controlled because the average molecular weights of the poly(L-lactide) tails should be related to the amine initiator concentration (i.e., average MW equals (grams of L-lactide monomer)/(moles of amine)).



Efforts are underway to optimize the concentration of amine, and the molecular weight of the poly(L-lactide), so that cobalt-poly(L-lactide) microspheres, which are high in cobalt concentration, can be prepared directly from these complexes. It is anticipated that this will lead to biodegradable poly(L-lactide) microspheres with higher magnetic response than will be possible with magnetite.

We have also been successful in functionalizing the silica-coated cobalt nanoparticle surfaces with other groups, including isocyanates and with poly(ethylene oxide) and polydimethylsiloxane oligomers [8].

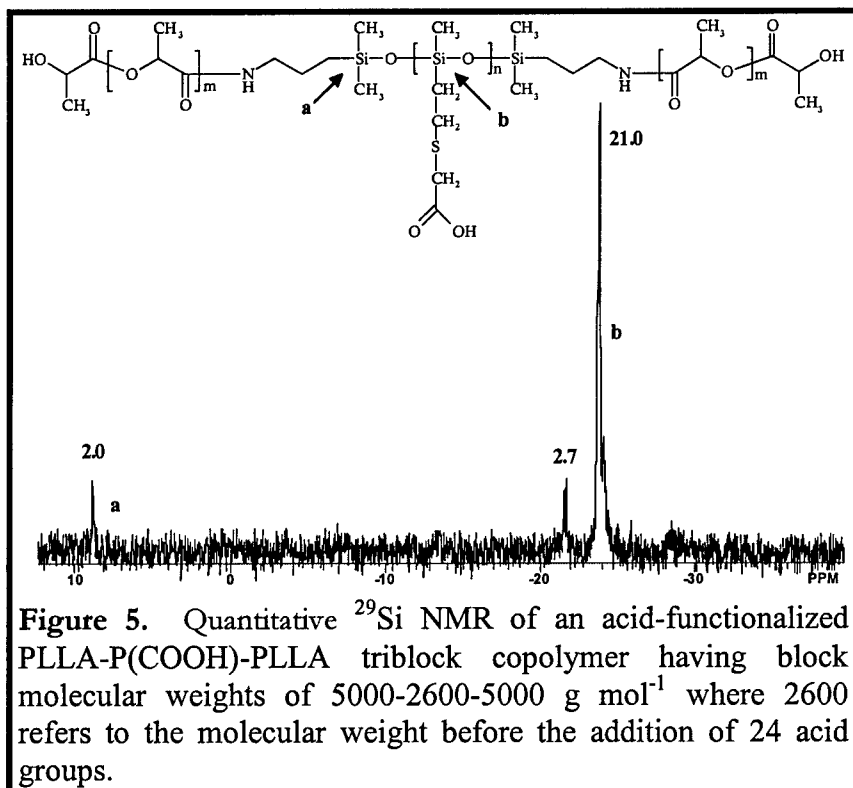
Magnetite-poly(L-lactide) microspheres.

We have discovered a general method for preparing complexes of magnetite with key triblock copolymers. The central blocks of the copolymers are functionalized with multiple carboxylic acid groups to bind with the magnetite surfaces [10-13]. The two end blocks of the copolymers remain free from the magnetite surfaces (i.e., they are not complexed with the magnetite). A family of triblock polymers with poly(L-lactide) end blocks and a central polysiloxane block containing carboxylic acid binding groups was developed. Both chemical block structures are based on biocompatible polymers since their intended applications are in-vivo.

The synthesis of the poly(lactide-siloxane-lactide) triblock copolymers comprises three reactions. First, difunctional, controlled molecular weight polymethylvinylsiloxane oligomers with aminopropyltrimethylsilyl endgroups were prepared in base catalyzed, ring-opening redistribution reactions. Second, these oligomers were utilized as macroinitiators for ring-opening L-lactide to provide triblock materials with polymethylvinylsiloxane central blocks and poly(L-lactide) end blocks. The molecular weights of the poly(L-lactide) end blocks were controlled by the grams of L-lactide relative to the moles of macroinitiator. Third, the vinyl groups on the polysiloxane center were functionalized with carboxylic acid groups by adding mercaptoacetic acid across the pendent double bonds in ene-thiol free radical reactions. ²⁹Si NMR spectroscopy was utilized to quantify the functional binding groups for the magnetite on the central anchor block (figure 5).

The triblock copolymers were complexed with magnetite nanoparticles that had been freshly prepared by co-precipitation of stoichiometric concentrations of FeCl₂ and FeCl₃ in aqueous hydroxide base. It was important that the pH be maintained at ~6 during complexation due to the extreme lability of the degradable polylactide esters in base. The magnetite nanoparticles had average radii of 4.8 nm measured by dynamic light scattering in water. The average surface area of the magnetite nanoparticles was measured by nitrogen absorption analysis (BET) to be 95 m² g⁻¹. A

representative procedure for preparing the magnetite nanoparticles and for complexing them with a polylactide copolymer is given below.



a pH meter. The pH was adjusted to ~ 9.5 by adding 10 mL of the NH_4OH solution while stirring. The solution immediately turned black indicating the formation of magnetite particles. The reaction mixture was stirred under N_2 for 60 min. The reaction was neutralized to pH ~ 6.0 -7.0 using a 4M HCl solution and allowed to continue stirring under N_2 for an additional 30 min. The appropriate amount of copolymer dissolved in 90% CH_2Cl_2 /10% MeOH v/v (25 mL) was injected into the reaction flask. The N_2 purge was removed and the reaction mixture was stirred for 18 h.

The reaction mixture was transferred to a one-neck, 250-mL, round-bottom flask. The reaction flask was washed with 90% CH_2Cl_2 /10% MeOH v/v to ensure the complete transfer of the product. The CH_2Cl_2 /MeOH solution was removed under vacuum and the remaining product was collected by holding a magnet to the bottom of the flask and decanting the water. The magnetite complex was washed with Millipore water three times to remove any salts. The complex was dried at room temperature, then redispersed in CH_2Cl_2 /MeOH solution (100 mL) and centrifuged to collect the nanoparticles. The nanoparticles were dried under vacuum and stored in a sealed 250-mL, one-neck, round-bottom flask.

The magnetite nanoparticles were coated with the acid-functionalized poly(L-lactide-*b*-siloxane-*b*-L-lactide) triblock copolymers. In the formation of the magnetite-copolymer complex, the pH was adjusted to pH ~ 6 with dilute HCl. In this pH range, the magnetite surface was slightly cationic (magnetite isoelectric point = pH 6.8) and the acid groups were partially ionized. Hence, this pH was utilized to promote adsorption of the carboxylate groups onto the magnetite surface. Once this was accomplished, the magnetite-copolymer complex was dispersible in chloroform or dichloromethane but not water. To confirm that the magnetite nanoparticles were coated with the copolymer stabilizer, the surface properties of the magnetite were analyzed using X-ray photoelectron spectroscopy. XPS analysis was also performed on magnetite coated with a 5,000-

Millipore water and a 50% aqueous solution of NH_4OH were deoxygenated by purging with N_2 for 30 min prior to the reaction. The reaction flask was purged with N_2 prior to the reaction to ensure an anaerobic environment. The iron salts were dissolved in 20 mL H_2O . Aqueous solutions of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (2.0 g in 20 mL H_2O , 0.389 M) and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (0.736 g in 20 mL H_2O , 0.195 M) were prepared separately under N_2 and syringed into a three-neck, 250-mL, round-bottom flask equipped with a mechanical stirrer and a pH electrode connected to

2,600-5,000 g mol⁻¹ triblock copolymer functionalized with an average of 24 acid groups per chain. Two complexes were prepared by charging 66 and 50 wt % magnetite.

XPS results confirmed that the nanoparticle surfaces were coated with the copolymer. The high iron (27 %) and oxygen (46%) on the surface of the bare magnetite particles suggested that they were relatively clean. Once the magnetite nanoparticles were coated with the copolymer, these surface compositions changed significantly. The iron content decreased from 27% to 2% suggesting that the particles were, indeed, coated with the triblock dispersion stabilizer. The presence of Si and S in the coated magnetite further confirmed the presence of the stabilizer on the surface. Thermogravimetric analysis (TGA) was utilized as a means for determining the efficiency of copolymer adsorption onto the magnetite nanoparticles. The nanoparticles were fractionated by centrifugation, then TGA and magnetic susceptometry were measured to probe the compositional distribution of polymer on the nanoparticles. Results suggested that although the nanoparticle surfaces were indeed coated, the amount of polymer on some of the particles was much higher than on others. These aspects will require further investigations to narrow the compositions over all of the particles.

Microspheres with diameters of ~2 microns and smaller were formed from these complexes in rapidly agitated dichloromethane-in-water suspensions. Poly(vinyl alcohol) was employed as the suspension stabilizer for the microsphere-dichloromethane droplets. The complexes readily disperse in solvents for the tail poly(L-lactide) blocks (e.g., dichloromethane), and this enables the formation of microspheres. It is encouraging that the size range of these microspheres is within that required for their introduction into the arterial system. However, the distribution of microsphere sizes remains relatively broad. Continuing work in this area will focus on (a) improving the homogeneity of the starting complexes, (b) understanding how the block lengths of the dispersion stabilizers translate into microspheres with homogeneous composition distributions, and (c) optimizing (increasing) the concentration of magnetite.

Work on these microsphere materials will be further coordinated in the upcoming year with Drs. Rosengart (Univ. Chicago) and Kaminski/Mertz (Argonne labs) for evaluation in both toxicity measurements and in forming hydrophilic, biotinylated surfaces.

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